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TITLE: Adult Tissue-Derived Stem Cells and Tolerance Induction in Nonhuman Primates for Vascularized Composite Allograft Transplantation

PRINCIPAL INVESTIGATOR: Eric A. Elster, MD

RECIPIENT: The Henry M. Jackson Foundation for the Advancement of Military Medicine

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CAPT Eric A. Elster, MD; Rolf Barth, PhD; Thomas Davis, PhD; Jeffery Gimble, MD, PhD

E-Mail:eric.elster@usuhs.edu

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Henry M Jackson Foundation for the Advancement of Military Medicine.
6720A Rockledge Drive, Suite #100, Bethesda, MD 20817-1834

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13. SUPPLEMENTARY NOTES

The utilization of adult derived adipose stem cells administration in composite tissue transplantation has shown positive results in multilineage T cell macrochimerism in rodents. This is a potential novel method of tolerance induction in humans, given the similarities to non-human primate models.

14. ABSTRACT

Amputations and unsalvageable injuries with devastating tissue loss are common in the combat wounded. Reconstructive transplantation in the civilian setting using vascular composite allotransplants (VCA) composed of multiple tissues (skin, muscle, nerve, bone) in combination with long-term multidrug immunosuppression has been encouraging. However, skin rejection remains a critical complication. We have demonstrated in a murine skin allograft transplantation model that human adipose-derived stromal cells (ASC) when used in concert with immunological conditioning support engraftment of limited numbers of donor bone marrow cells (dBMCs) across major histocompatibility complex (MHC) barriers, and lead to stable multilineage mixed-chimerism and skin allograft tolerance without the need for long-term immunosuppression.

Focus Areas: Immune Rejection-understanding mechanisms of immune rejection, immunomodulation approaches and mechanisms (e.g., tolerance induction, chimerism), and optimizing immunosuppressive drug regimens.

Objectives/Hypothesis: We realize that the implications and potential clinical benefit of the tolerance induction protocol to shown efficacy in a mouse model can only be validated mechanistically in established non-human primate models of allograft transplantation with long-term observations and evaluations. We hypothesize that ASCs+dBMC therapy may be a pro-tolerogenic cellular therapeutic displaying clinical efficacy for vascular composite allograft (VCA), solid organ, and hematopoietic stem cell transplant applications. This combination would allow for long term graft survival without the need for chronic immunosuppression and the resulting multitude of adverse effects associated with such agents.

Specific Aims: (1) To investigate whether ASCs augment chimerism and promote long-term VCA graft survival and (2) To determine whether ASC therapy allows for immunosuppression minimization and development of immunologic tolerance to VCA.

Study Design: We will use a non-human primate (NHP) model for facial vascular composite allografts (VCA) utilizing cynomolgus macaques that has demonstrated reproducible technical success over the last 10 years. Recipients will receive a VCA transplant on day 0 and then treated with the experimental immunosuppressive regimen. Non-myeloablative conditioning (anti-CD4/CD8 days 0-14, busulfan on day 5) and ASC + dBMC.

15. SUBJECT TERMS					
Vascularized composite allotransplantation (VCA), calcineurin inhibitors (CNI), adipose-derived stem cells (ASC), Non-human primate (NHP), tolerance					
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1. INTRODUCTION:

Vascularized composite allotransplantation (VCA) have demonstrated graft survivals over a decade with conventional immunosuppression based on calcineurin inhibitors (CNI) have defined rates of renal failure, diabetes, and other side effects. Increasing the application of VCA to wounded military personnel depends on strategies that will safely extend graft survivals to many decades. This will be accomplished by immunosuppressive strategies that protect both the graft (decreased rejection) and the patient (decreased complications). Our studies investigate whether utilizing adipose derived stem cells (ASC) in these transplants can reduce or eliminate (tolerance) the need for immunosuppressive medications and associated toxicities. Ultimately, these experiments will answer the important question of whether the use of ASCs and low numbers of donor-derived bone marrow cells can promote the development of chimerism and tolerance, and represent an improved immunosuppressive strategy for VCA.

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

Vascularized composite allotransplantation (VCA), calcineurin inhibitors (CNI), adipose-derived stem cells (ASC), Non-human primate (NHP), tolerance

3. ACCOMPLISHMENTS:

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Task 1 (100% completed)

Subtask 1: Submit documents for University of Maryland, Baltimore Institutional Animal Care and Use Committee (IACUC) approval. (completed 01OCT2016)

Milestone # 1 IACUC approval obtained (completed 19OCT2016)

Subtask 2: Submit documents for ACURO approval (approved Feb 22, 2017)

Milestone#2 ACURO approval obtained (100% completed)

Subtask 3: Contract/Order of 8 NHP for Aim 1 experiment

Milestone # 3 NHP received to Site 1 (100 % completed)

Subtask 4: Initiate 90 day quarantine, perform immunogenetic testing to select donor/recipient pairs for 4 experiments (100% completed).

Milestone #4 Completion of 90 day quarantine and selection of experimental pairs (100% completed)

Major Task 2 (0% completed)

Subtask 1: Surgical procedure of facial VCA transplant [(1 donor + 1 recipient) X 4 groups = 8 NHP total]

- Coordination and delivery of prepared cell products to UMB
- 30-day follow-up including clinical course and in vitro assays

Milestone #5 Completion of Aim 1 experiments

Subtask 2: Assess primary outcomes of 30-day graft survival and chimerism

- Data analysis and interpretation of diagnostic assays performed at UMB and USUHS including cell products, flow cytometry, laboratory data, graft biopsies

Milestone #6 Completion of chimerism studies and laboratory assays

Major Task 3 (0% completed)

Subtask 1: Immunosuppression weaning to low-level tacrolimus monotherapy (Day 31-90)

- Follow-up including clinical course (rejection, laboratory data, animal health assessments) and chimerism assays (flow cytometry data)

Milestone #7 Completion of immunosuppression weaning

Subtask 2: Stop immunosuppression to test for development of immunologic tolerance

- Follow-up including clinical course (rejection, laboratory data, animal health assessments) and chimerism assays (flow cytometry data) and data analysis to determine success of approach

Milestone #7 Completion of immunosuppression cessation

Subtask 3: Donor skin and 3rd party skin grafting to assess for donor-specific tolerance

Milestone #8 Completion of skin graft and immunologic assays

What was accomplished under these goals?

Accomplishment 1: Amended ACURO proposal approved on 22FEB2017

Accomplishment 2: 8 cynomolgous monkeys were purchased and received at Site 1 on 22FEB2017. They have commenced with the 90-day quarantine and are expected to clear quarantine on 22MAY2017. They have currently cleared quarantine

Accomplishment 3: We have developed methods to isolate, *ex vivo* propagate, functionally characterize, and cryopreserve (biobank) non-human primate adipose-derived stem cells (ASCs) derived from non-human primates. Adipose tissue was harvested from euthanized non-human primates housed the University of Maryland and the Tulane University National Primate Research Center are being used for ASC isolation, characterization and cryopreservation for future use in our vascular composite allograft transplant studies. It is estimated that each animal recipient will require approximately 100 million culture expanded ASCs.

Results: The past funding period is noteworthy for the following accomplishments: (1) Cultures were established with SVF cells isolated from rhesus macaque adipose tissue obtained from necropsies performed at Tulane National Primate Research Center. Comparison in ASC cell growth was compared using LaCell's Stromal Medium (SM), 50% fresh SM supplemented with 50% conditioned medium from non-human primate ASC (CM), or 50% SM: 50% CM further supplemented with 0.75% human platelet lysate. Based on cell expansion, all medium compositions performed equally well and LaCell SM is now used without supplement. (2) Using 5 grams of subcutaneous adipose tissue obtained from the University of Maryland (AF692K), SVF cells were expanded over 5 consecutive passages. From an initial yield of 0.8 X 106 cells obtained after 24 days in culture, the successive passages of ASC resulted in subsequent fold expansions of: 2.75X (4 days), 7.2X (10 days), 59X (11 days), 1.9X (5 days), 2.8X (5 days). Thus, in 2 months of culture, we conservatively estimate that it will be feasible to obtain 688-fold expansion of the initial Passage 0 population which should be more than sufficient for the ongoing study.

Accomplishment 4: We have identified the need to test depletional antibodies and busulfan in 1-2 NHP. This is necessary to confirm dosing and efficacy of the proposed drugs. Potential toxicities needed to be identified prior to commencing with the experimental group. In order to test these reagents in vivo, additional IACUC amendment and ACURO approval was necessary. IACUC approval was received from University of Maryland on 5 OCT 2017. ACURO submission is in preparation

What opportunities for training and professional development has the project provided?

The laboratory has been very active in the training of research fellows and young faculty. During the period of funding support, three research fellows participated in funded studies. One fellow has returned to completing surgical training with intention to apply for clinical fellowships in plastic and reconstructive surgery.

Our laboratory's overall record of accomplishment overall has resulted in training 12 graduate and post-doctoral fellows and 8 undergraduates in transplant immunology and reconstructive transplantation. They have been trained in surgical techniques of transplantation in animal models, large animal handling, immunological lab techniques, assistance with care and evaluation of clinical VCA patients, as well as the logistics of running a large animal lab. Four of these fellows have completed plastic surgery fellowships and three have academic appointments as faculty in plastic and reconstructive surgery. Two prior fellows are completing clinical fellowships in cardiothoracic surgery and plastic surgery.

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

Our plan is to obtain sterile cynologus macaque subcutaneous adipose tissue directly from the University of Maryland or its affiliated animal resource facilities in South Carolina and culture expand all isolated SVF cells through at least Passage 3 to create a biobank of ASCs cryopreserved in aliquots of 20 X 10^6 cells per 1 ml of cryopreservation medium. An individual cryopreserved vial of ASC from this lot will be characterized based on viability at time of thawing, immunophenotyping with a panel of antibodies, and differentiation characterization based on adipogenesis and osteogenesis.

Test the immunodepletional agents and the immunodepletional strategy in 1-2 naïve NHPs prior to commencing with VCA experiments.

Recipient T cell populations will be depleted with anti-CD4 and anti-CD8 antibodies on days 0, 2, 4, 7, 14 and given a single non-myeloablative infusion of busulfan on day 5. Peripheral blood will be collected periodically to document the change in T cell population post treatments. One week post-transplant four animals will receive an infusion of purified fat-derived stem cells. Soft tissue biopsies and flow cytometry will be collected to assess graft status and chimerism. The effects on graft survival and macrochimerism will be the primary data shared.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

The utilization of adult derived adipose stem cells administration in composite tissue transplantation has shown positive results in multilineage T cell macrochimerism in rodents. This is a potential novel method of tolerance induction in humans, given the similarities to non-human primate models.

What was the impact on society beyond science and technology?

Immunosuppression required at time of transplantation, such as calcineurin inhibitors, can lead to a number of side effects, including renal failure, infection, and malignancy. These present significant health risks, mortalities, and expenses to patients and their families, hospital systems, and society at large. Establishing a tolerance regimen has the potential to provide longer lasting and more cost efficient method to protect transplanted organs and tissues.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

We have identified the need to test depletional antibodies and busulfan in 1-2 NHP. This is necessary to confirm dosing and efficacy of the proposed drugs. Potential toxicities needed to be identified prior to commencing with the experimental group.

Actual or anticipated problems or delays and actions or plans to resolve them

In order to test these reagents in vivo, additional IACUC amendment and ACURO approval was necessary. IACUC approval was received from University of Maryland on 5 OCT 2017. ACURO submission is in preparation. The need to test the reagents in 1-2 NHP have affected expenditures with drug and animal purchasing.

The major change to the approach is that we will now consider expansion of the ASC for extended periods of time and through multiple successive passages. While we initially had expected to obtain a sufficient number of ASC directly from a single passage of the adipose tissue-derived SVF cells, we have recognized that this is not tenable due to the small amount of subcutaneous adipose tissue available in most non-human primates.

The major problem that we have faced has been contamination of specimens, possibly at the time of harvest, in transit, and/or during the culture expansion period. We have addressed this by developing a more potent cocktail of bacterial and fungal inhibitors which is now being used as a culture medium supplement. Additionally, concentrated antibiotics and antimycotics are being added to the medium during the shipping period.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals.

IACUC amendment to test CD4, CD8, and busulfan in up to 2 animals to test drug efficacy. Approved 5 OCT 2017.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers, and presentations.

Barth RN. Immune Pathways to New Operations. Department of Surgery Grand Rounds, University of Vermont Larner College of Medicine; June 29, 2017: Burlington, VT.

Barth RN. VCA Transition from Research to Standard of Care. VCA Transplant Ethics Meeting at NYU School of Medicine; July 11, 2017: New York, NY.

Barth RN. Immunosuppressive Protocols in Transplantation. Evolving Issues of Vascularized Composite Allo-transplantation; Johns Hopkins Mt. Washington Conference Center; September 19, 2017; Baltimore, MD.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Eric A. Elster, MD
Project Role: Principal Investigator
Nearest person month worked: 1
Contribution to Project: Provides oversight of the entire project development and implementation all
policies, procedures, and processes.

Name: Rolf Barth, PhD
Project Role: Co-Principal Investigator
Nearest person month worked: 1
Contribution to Project: Provides oversight of the entire project development, serves to ensure completion
of the specific aims on the schedule proposed and scientific quality control for animal study analysis, an
troubleshoots technical issues that may arise in the day-to-day management of the project.

Name: Thomas A. Davis, PhD
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to Project: Assists with project development, serves to ensure completion of t
specific aims on the schedule proposed and scientific quality control for animal study analysis, and
troubleshoots technical issues that may arise in the day-to-day management of the project..

Name: Jeffrey Gimble, MD, PhD
Project Role: Research Collaborator
Nearest person month worked: 1
Contribution to Project: Isolation, ex vivo expansion, functional characterization and the cryopreservation
of non-human primate adipose-derived stem cells.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Not Applicable.

Adult Tissue-Derived Stem Cells and Tolerance Induction in Nonhuman Primates for Vascularized Composite Allograft Transplantation

Congressionally Directed Medical Research Programs - W81XWH-16-2-0042

PI: Eric Elster, Co-PI Rolf N. Barth **Org:** The Henry Jackson Fdn. for the Advancement of Mil. Med., Inc.

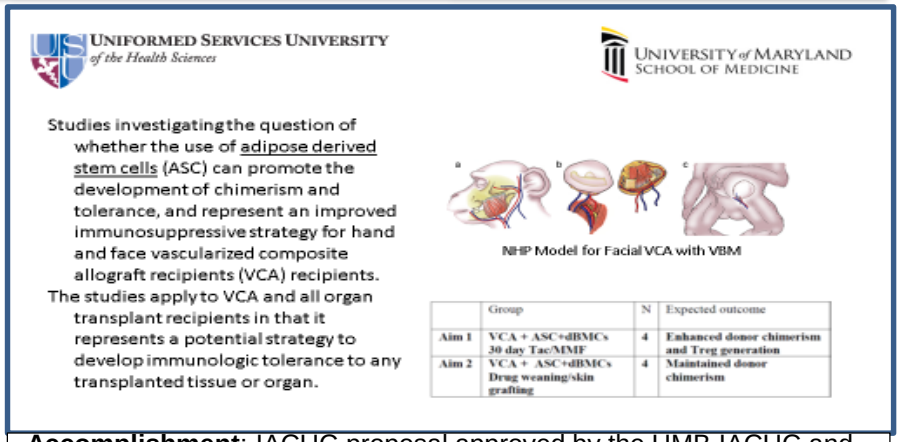
Work Perform at: Uniformed Services Univ. of the Health Sciences/Univ. of Maryland **Award Amount:** 448,415

Study/Product Aim(s)

- Our studies investigate whether utilizing adipose derived stem cells (ASC) in hand/face vascularized composite allograft (VCA) transplants can reduce or eliminate (tolerance) the need for immunosuppressive medications and associated toxicities in service members who would benefit from these reconstructive approaches to devastating facial and limb injuries.
  - Aim 1. To investigate whether ASCs augment chimerism and promote long-term VCA graft survival.
  - Aim 2. To determine whether ASC therapy allows for immunosuppression minimization and development of immunologic tolerance to VCA.

Approach

Non-human primate (NHP) experiments in our established model of facial VCA to examine the ability of ASC establish chimerism, promote graft survival, and establish immunologic tolerance.



Accomplishment: IACUC proposal approved by the UMB IACUC and ACURO. NHP ordered, received and completed 90-day quarantine period. Continue with isolation, propagation, cryopreservation and functional characterization on non-human primate adipose-derived stem cells. Commenced with normal skin imaging analyses.

Timeline and Cost

Activities CY	16	17	18
ASCs augment chimerism in NHP facial VCA			
ASC therapy allows for immunosuppressive therapy reduction and withdrawal in NHP facial VCA			
Estimated Budget ($448K)	**0**	**280**	**168**

Updated: October 18, 2017

Milestones/Goals

CY16 Goals

- ✓ Approved UMB IACUC
- ✓ Approved DOD ACURO

CY17 Goals

- ✓ NHP animals screened, selected, and delivered to UMB site
- ❑ 4 completed transplants with up to 30-day follow-up

CY18 Goals

- ❑ 4 experimental animals weaned to low level immunosuppression for pending cessation after facial transplant
- ❑ 4 animals with completed skin grafts and determination of tolerance.
- ❑ Complete follow up of all NHP experiments

Budget Expenditure to Date:
Actual Expenditure: $51,646.71